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Short communication

Study of the inclusion complex of atenolol with β-cyclodextrins☆

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1. Introduction

Poor bioavailability is, unfortunately, a frequent problem for drug delivery. Many molecules that are biologically active in vitro are inactive in vivo as a result of a variety of possible problems such as limited solubility or stability, adverse side effects, and limited transport across cell membranes.

Many works can be found in the literature regarding the low bioavailability of insoluble and unstable drugs. Among the different methods proposed to overcome this problem, the molecular encapsulation of these drugs by cyclodextrins (CD) is probably the most widely used [1–7]. Their toroidal structure, with hydrophilic external face and hydrophobic inner surface, makes them the most important simple organic compounds capable of forming, by non-covalent bonding, as in Van der Waals interactions, hydrophobic effect, solvent reorganization and hydrogen bonding [8], host–guest systems more soluble and stable.

On the other hand, drugs that are highly polar and soluble in aqueous medium can also have poor bioavailability as a result of inefficient transport across the hydrophobic lipid bilayer constituting the cellular membranes.

'Molecular encapsulation' by means of monomolecular inclusion complexes formation has offered promise for the development of new dosage forms and its importance in pharmaceutical formulations has been fully realized [9]. Generally, the inclusion complex involves the spatial entrapment of a single guest molecule in the cavity of one host molecule without the formation of any covalent bonds. This is the essence of the so-called molecular encapsulation [10,11].

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As in recent years the β-blockers were described as one the most important pharmacological and therapeutic innovations [12–14], our interest has been turned to these compounds. The aim of this study was to increase the solubility and dissolution rate of atenolol by inclusion complex formation with β-cyclodextrin (β-CD).

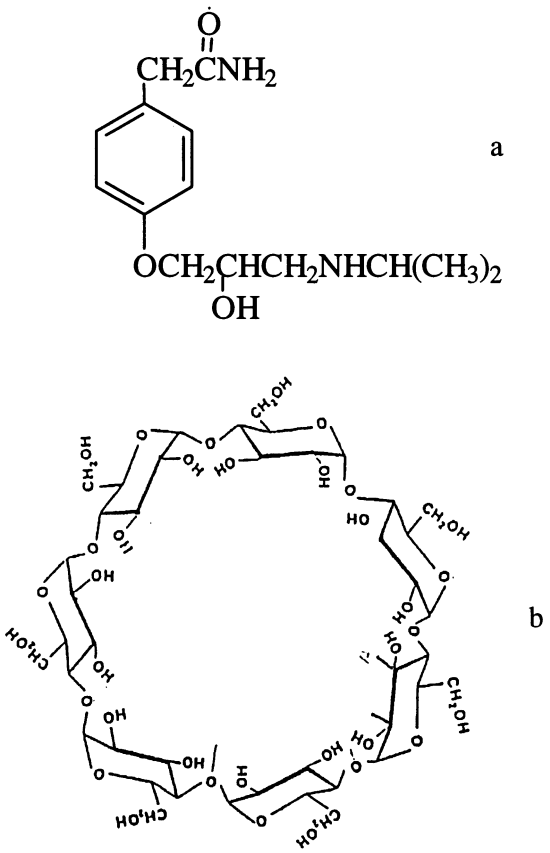

Fig. 1. (a) Atenolol; (b) β-cyclodextrin.

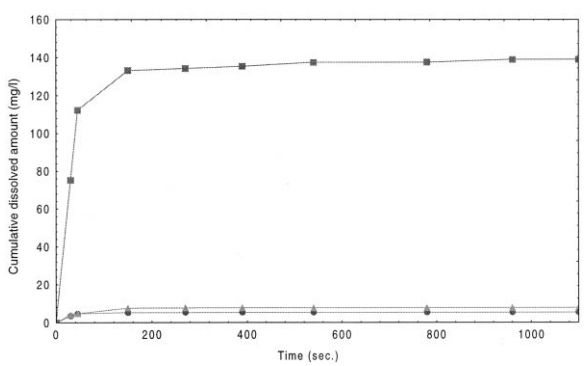

Fig. 2. Phase solubility diagram for atenolol with β-CD at 25°C.

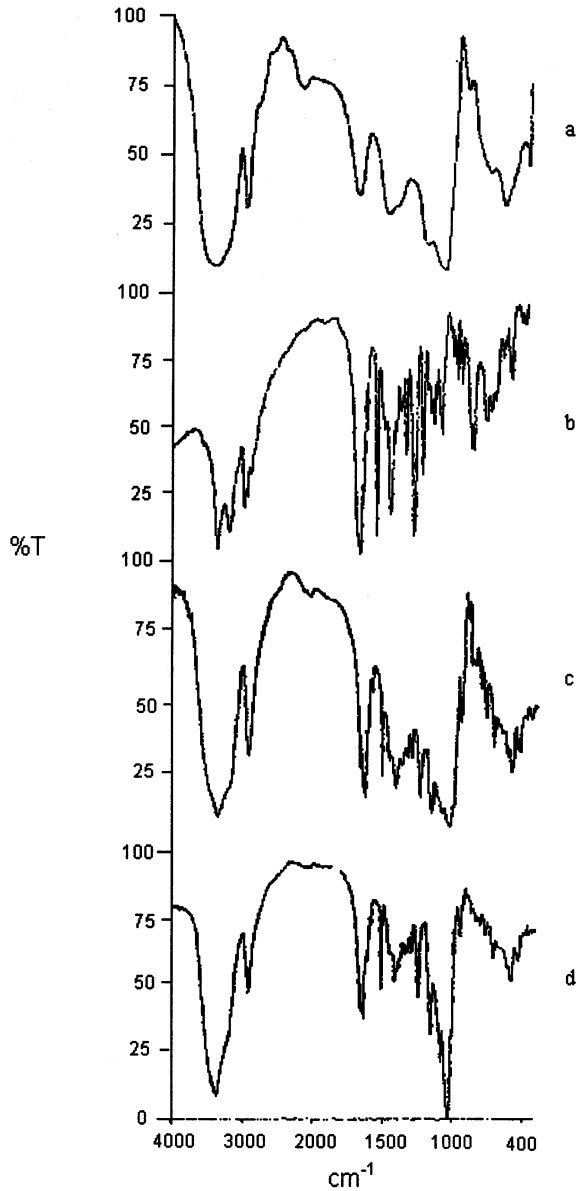

Fig. 3. FT-IR spectra of the atenolol/β-CD system. (a) β-CD alone; (b) pure atenolol; (c) physical mixture of atenolol and β-CD; (d) atenolol/β-CD complex.

2. Materials and methods

2.1. Materials

The β-blocker atenolol (Fig. 1(a)) was supplied by Sigma-Aldrich Chemie (Germany). The β-cyclodextrins (Fig. 1(b)) were provided by Fluka Chemie (Switzerland). Potassium bromide was provided by Sigma Aldrich. Solutions at different pH values (0.5, 1.5, 3.0, 7.0 and 8.0) were prepared. The hydrogen ion concentration was obtained at 25°C, according to F.U. X, by using solution of HCl 0.1 M and KCl 0.1 M (pH 1.5), sodium phosphate dibasic 0.2 M and citric acid 0.1 M (pH 3.0, 7.0, 8.0), pH values refer to a temperature of 25°C. The water used for solutions was distilled, deionized and filtered through a 0.22 μm Millipore Filters (Bedford, USA). All other materials were of analytical reagent grade.

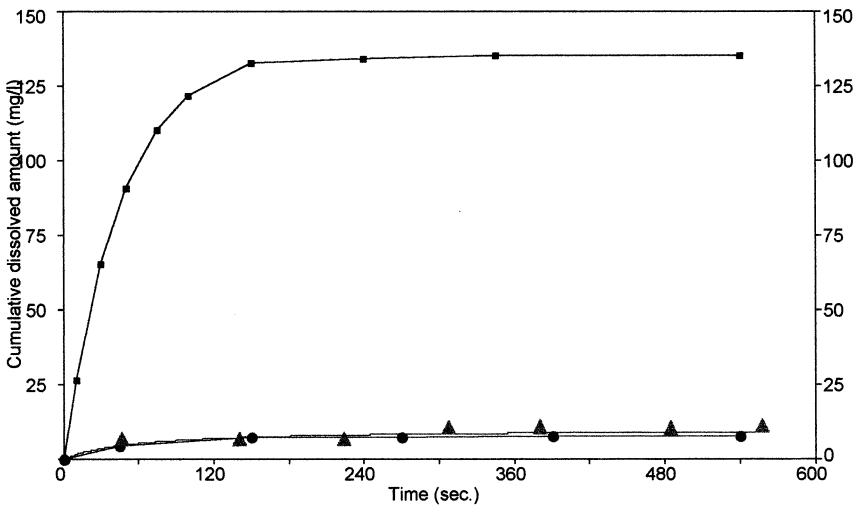

Fig. 4. Dissolution profile at 37°C of atenolol and atenolol/β-CD system at pH 3.0. (●) pure atenolol; (▼) atenolol/β-CD physical mixture; (■) atenolol/β-CD co-precipitated complex.

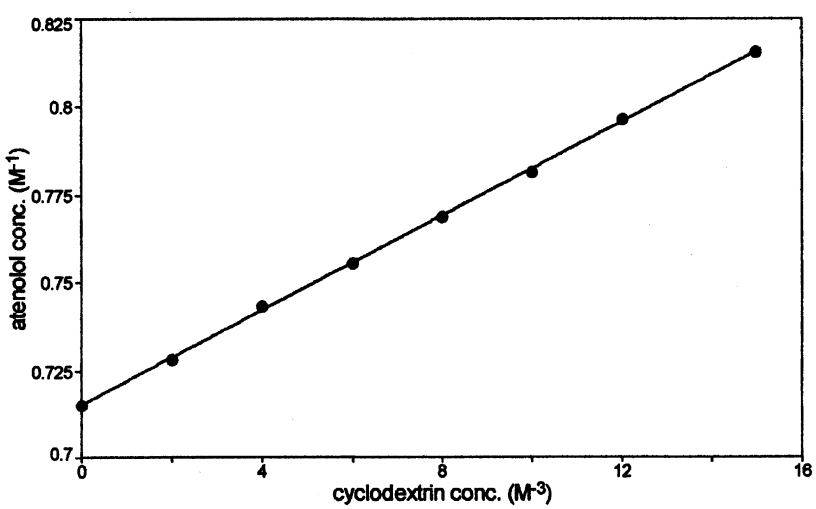

Fig. 5. Plot of cyclodextrin concentration versus atenolol concentration.

2.2. Apparatus

The dissolution rate studies were carried out using the F.U. X paddle method. The dissolved drug was quantified using a Perkin-Elmer Lambda 3 spectrophotometer connected to a P II 350 MHz computer and equipped with a cell compartment thermostated by a Perkin-Elmer PTP (Peltier Temperature Programmer) which allows a controlled change of the temperature with an accuracy of ± 0.05°C. The absorbance–time data were acquired by using the Perkin-Elmer PECSS program.

Processing of data for the fitting was done using Jandel Scientific PEAKFIT 4.0 program with the Marquardt algorithm. An IR spectrophotometer (FTIR Perkin-Elmer 720-X) interfaced to a Pentium II 350 MHz processor was used for the analysis IR.

2.3. Preparation of the samples

The complex of the atenolol with the β-CD in 1:1 molar ratio was prepared by the co-precipitation method. Atenolol (32 mg) and β-CD (136.4 mg) were added to 20 ml of water, sealed in a flash and the mixture was stirred at room temperature for 24 h. Then the solution was dried under reduced pressure. The mode of preparation of the physical mixture was the simplest. The calculated and exactly weighed (1:1 molar ratio) amounts of atenolol and β-CD were powdered in a mortar and carefully mixed. Previously powdered and sieved (particle size lower than 100 μm) samples (150 mg of atenolol or equivalent amount of binary systems) were used for all dissolution studies.

2.4. Phase solubility studies

The phase solubility studies were carried out according to the method reported by Higuchi and Connors [15]. Excess amounts of the atenolol (400 mg) were weighted into 10 ml tubes, to which were added 10 ml of aqueous solutions containing various concentrations of β-cyclodextrins (0.001–0.015 M) and shaken at 37 ± 0.5°C.

At the equilibrium after 24 h, an aliquot was filtered through a Gelman Science Acrodisc® LC PVDF 45 μm filter. A portion of the sample was adequately diluted and analyzed spectrophotometrically to determine the concentration of atenolol. The experiment was carried out in triplicate.

The apparent formation constant, K_c was calculated from the straight-line portion of the phase solubility diagram according to the following Higuchi–Connors equation:

$$K_c = \frac{\text{slope}}{\text{intercept}(1 - \text{slope})}$$

2.5. Fourier-transform infrared spectroscopy (FTIR)

The IR spectra of the atenolol and complex were measured as potassium bromide discs. For comparison, the IR spectra of atenolol/β-CD physical mixture and pure β-CD were carried out using the same procedure.

2.6. Dissolution test

The duration of the assay was 1 h and samples were withdrawn at measured time intervals and filtered with a Gelman Science Acrodisc® LC PVDF 45 μm filter. Dissolved drug was assayed at variable wavelengths according to the pH of the buffer solution (226 nm at pH 8.0, 228 nm at pH 7.0, 238 nm at pH 3.0 and 225 nm at pH 1.5) in a Perkin-Elmer Lambda 3 spectrophotometer and three replicates of each dissolution assay were carried out. Processing of data for the fitting was done using Jandel Scientific PEAKFIT 4.0 program with the Marquardt algorithm.

3. Results and discussion

3.1. Phase solubility study

The phase solubility diagram corresponding to the atenolol/β-CD system is reported in Fig. 2. The solubility of atenolol increases linearly as a

function of β-CD concentration ($0–15 \times 10^{-3}$ M) and the solubility curve can be classified as type A_L at the temperature of 25°C [15]. When there is a linear increase in drug solubility with increasing cyclodextrin concentration, a cyclodextrin complex of drug results from 1:1 mol/mol interaction. Accordingly, we can assume that a 1:1 mol/mol atenolol/β-CD inclusion compound was formed and this is in good agreement with that obtained by isolation and analysis of the crystalline complex [16]. The apparent association constant at 25°C was calculated from the slope and intercept of the A_L solubility diagram as 28.66/M according to the Higuchi–Connors equation.

3.2. FTIR (Fourier-transform infrared spectroscopy)

The complex of the atenolol with the β-CD was examined by IR spectroscopy measurement and compared with the pure atenolol, the pure β-CD and the corresponding physical mixture in the same molar ratio. Fig. 3 shows IR spectra of atenolol, β-CD, atenolol/β-CD physical mixture and atenolol/β-CD complex. As shown in the figure, atenolol has a carbonyl band of 1725–1685 cm^{-1}. In the IR spectrum of the physical mixture there is no change. Whereas, in the IR spectrum of the corresponding complex, prepared by co-precipitation method, there is a change in its carbonyl band and a significance decrease was observed in its intensity. These spectral changes can be explained by the dissociation of the intermolecular hydrogen bounds of the atenolol trough inclusion complexation. The observed decrease in intensity of the carbonyl band may have resulted from its restriction within the β-CD cavity.

3.3. Dissolution study

The above results clearly indicate that the atenolol/β-CD complex, which was prepared by the co-precipitation method, exists in the solid state. The dissolution rate profiles of complexes, physical mixture and pure atenolol in a buffer solution at pH 1.5 show that the co-precipitated complex exhibits a little faster dissolution rate than the atenolol and the physical mixture. The dissolution rate profile of complex in the buffer solution at pH 3.0, as seen in Fig. 4, shows a greater and faster dissolution compared with the pure atenolol and the physical mixture, that at pH 3.0 are sparingly soluble. In co-precipitated complex, an increase was observed in the dissolution rate within 3 min. Later, it reached an asymptotic level. The dissolved amount of the atenolol was 0.5% while the same amount were 0.6 and 91% respectively, in the physical mixture and the co-precipitated complex. The dissolution profiles at pH 7.0 and 8.0 show that dissolution rate of the pure atenolol, the physical mixture and the complex is too rapid for the evaluation.

4. Conclusions

This study shows that there is formation of a β-CD/atenolol complex in aqueous solution and this complex, prepared by the co-precipitation method, also exists in the solid state. Association constant and stoichiometric ratio have been calculated by phase solubility study and from the results we can assume that the formation of the complex association of both atenolol and CD can increase the aqueous solubility of atenolol. The improved dissolution rate may be as a result of the increase in solubility, brought about by complexation. From the results we can assume that the aqueous solubility and dissolution rate of atenolol can be significantly increased by forming an inclusion complex with β-CD (Fig. 5).

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